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# NUTRITION REVIEWS

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# NUTRITION REVIEWS

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## FOOD COMPOSITION AND NUTRITION PROGRAMS

The usefulness of data on the composition of foods in relation to the food and nutrition problems of any nation may be demonstrated by drawing from experiences in Mexico and in Central America during recent years.

A nutrition survey of the Otomi Indians in the Mezquital Valley of Mexico showed that pronounced clinical nutritional deficiency was not common (R. K. Anderson, J. Calvo, G. Serrano, and G. C. Payne, *Am. J. Pub. Health* **36,** *883 (1946); Nutrition Reviews* **4,** *347 (1946)*) yet these people were eating very few of the foods which are usually considered essential to a good nutrition pattern. Because this region is arid and the soil barren, consumption of meat, dairy products, fruits, and vegetables was extremely low. Instead, they were consuming tortillas, pulque (fermented juice of the century plant), and numerous edible plants growing in the area, such as malva, hediondilla, tuna, nopales, maguey, garambullo, yucca, purslane, pigweed, sorrel, wild mustard flowers, lengua de vaca, sow thistle, and cactus fruits.

At first it was not possible to estimate the adequacy of the Otomi diet on the basis of food intake because the nutrient content of most of the foods composing it had never been measured. Later, samples of those foods commonly eaten were frozen and shipped in carbon dioxide ice to the Massachusetts Institute of Technology for chemical analysis (R. O. Cravioto *et al., J. Nutrition* **29,** *317 (1945)*). Seven day records of the food consumption of the inhabitants of four villages were then obtained and the nutrient content of the diets calculated from the data obtained by chemical analysis. The average daily intake of certain essential nutrients was compared with the Recommended Allowance (*National Research Council, Reprint and Circular Series* **122** *(1945)*), with the following results (in per cent): calories 70, protein 80, calcium 85, iron 203, vitamin A 127, thiamine 135, riboflavin 42, niacin 79, and ascorbic acid 141. The intake was superior to that of an urban group in the United States studied at the same time (E. E. Lockhart *et al., J. Am. Dietet. Assn.* **20,** *742 (1944)*). It appears that the nutrition of the Otomi Indians should be improved, not by changing their food habits to conform with the pattern of the United States, but by making better use of foods indigenous to the Mezquital area.

Another example of the usefulness of data on the composition of foods is found in our attempts to develop a school lunch program in collaboration with Dr. Francisco deP. Miranda and the Mexican Institute of Nutrition. A preliminary survey indicated that many of the foods commonly eaten in Mexico were unknown in the United States, and that few of them had been analyzed for nutrient content. A practical, nutritious school lunch could not be planned without data on the composition of the common, inexpensive foods available in the area. When these data were obtained (Cravioto *et al., loc. cit.; Science* **102,** *91 (1945)*) it was possible to compound a series of lunch menus which supplied in each meal one third of the N. R. C. allowance at 20 per cent of the cost of a lunch based on the United States' food pattern. It has been remarked elsewhere (R. S. Harris, *Ibid.* **102,** *42 (1945)*) that there are many parts of the world where the American pattern of good nutrition should not be advocated because the foodstuffs which assure good nutrition for us are not often those which can most effectively and economically nourish the people of other countries.

Recently a program was undertaken in cooperation with the Escuela Agricola Panamericana, in which the composition of the foods of Middle America is being studied.

This endowed agricultural school is located in Honduras and offers a three year course to the young men of Middle America. The director, Dr. Wilson Popenoe, wished to add a course in nutrition to the curriculum. When it was pointed out that it would not be possible to teach nutrition until data were available on the composition of Middle American foods, a food analysis program was planned so that these data might be obtained.

A resident botanist is employed to make observations on the food habits of the people, collect specimens for botanical identification, and take samples for chemical analysis.

TABLE I

*Comparison of the nutrient content of plant foods in Middle America and the United States*

| | Minimum value as basis of comparison (/100 g.) | Number of plant foods above minimum value | | Number of Middle American foods above United States' maximum |
|---|---|---|---|---|
| | | United States | Middle America | |
| Nitrogen | 2.5 g. | 8 | 20 | 1 |
| Calcium | 100.0 mg. | 12 | 39 | 6 |
| Iron | 3.0 mg. | 19 | 51 | 10 |
| Carotene | 2.0 mg. | 14 | 32 | 3 |
| Thiamine | 0.2 mg. | 16 | 42 | 5 |
| Riboflavin | 0.2 mg. | 13 | 34 | 1 |
| Niacin | 2.0 mg. | 13 | 31 | 0 |
| Ascorbic acid | 75.0 mg. | 7 | 48 | 20 |

These samples are shipped by air to the Massachusetts Institute of Technology and here analyzed. It is hoped that analyses will be completed during the next three to five years on samples of all food plants used by the people of Middle America. In many instances samples of the same plant will be taken from several areas so that the effects of climate and soil, as well as genetics, may be studied.

During the first year of this program, more than two hundred food samples have been analyzed. These results together with those obtained on samples of Mexican foods indicate that there are more foods rich in each of the essential nutrients in Latin America than in the United States. Table I was prepared from data obtained on samples analyzed in the Massachusetts Institute of Technology laboratories, and from data presented in Bulletins **572** and **549** of the United States Department of Agriculture, the two most reliable summaries of data on the composition of United States foodstuffs. The high nutritive value of these foods may be an important reason for the relatively low incidence of malnutrition observed in a group of 1000 impoverished schoolchildren (R. S. Harris, *J. Am. Dietet. Assn.* **22,** *974 (1946)*).

A significant percentage of the foods in Middle America is unknown in this country and many of them have never before been analyzed. Furthermore, some of the foodstuffs grown in the United States are less nutritious than the same strains grown in Middle America. This is probably the result of a combination of factors, among them genetics, soil, and climate differences. Increased yields in the United States have often been obtained at the expense of nutritive quality.

Among the foods analyzed, one, Mammea americana, gives evidence of containing an antibacterial substance, for it inhibits the growth of the Lactobacillus arabinosus organism in the microbiologic assay for niacin. It has been reported that this fruit is used by the Indians to combat infestations and infections. It would be interesting to determine the extent to which this and other foods with antibacterial properties, interfere with the intestinal synthesis of vitamins. It is evident that data from the microbiologic assay of an unusual plant species should be accepted as tentative until it has been established that it contains no interfering substances.

After the composition of the edible foods in an area has been determined by analysis of many samples gathered from different locations and at different seasons, a sound food and agriculture program may be developed. The farmer can then develop crops which yield more nutritious food and the

nutritionist can advocate a greater consumption of those foods which give the most economical nourishment.

Although the food chemist should first determine the nutrient content of foods taken from the ground and from the market place, he should not neglect a study of the nutrient content of the diets as eaten by the people. Nutritious substances (for example, condiments, spices, and lime) are often used in the preparation of food and these may escape analysis.

In Latin American countries pulverized limestone is used in the preparation of the tortilla from corn and this raises the calcium content from 9 mg. per cent in corn to 190 mg. per cent in the tortilla (Cravioto *et al.*, *Science* **102,** *91* (*1945*)). Analyses of dietaries will reveal also the extent to which cooking practices destroy unstable nutrients so that improvements in culinary procedures may be suggested.

It appears that the food problems of other nations will not be solved by imposing our foods or food habits upon them. A long step can be made toward a practical solution of the nutrition problem by an analysis of the food plants and dietaries of each area, and by the production and consumption of the most nutritious indigenous foods. In this sense food analysis is basic to the solution of the nutrition problems of the world.

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## WHY DOES PARBOILING INCREASE THE THIAMINE OF RICE?

L. Nicholls (*Nature* **160,** *298* (*1947*)) has raised the question of why rice which is parboiled before it is milled, possesses the power of banishing beriberi from the population which subsists on it.

It has generally been accepted that parboiling distributes the vitamins of the pericarp and germ throughout the whole grain, so that milling can remove "only a small part" with the bran. Many analyses have shown that parboiled rice is richer in thiamine than raw milled rice, which agrees with the experience that beriberi is rare where parboiled rice is the staple article of diet.

It has long been known that the thiamine of cereals is not evenly distributed throughout the grain but is mainly concentrated in the outer coats of the grain and in the germ, consisting of the embryo and the attached scutellum. J. J. C. Hinton (*J. Soc. Chem. Ind.* **61,** *143* (*1942*)) dissected wheat grains carefully so as to separate the different structures, and then determined the thiamine content of each. He found that there was an exceptionally large concentration in the scutellum; although on the average it formed only 1.54 per cent of the grain it contained 59 per cent of the thiamine of the grain. It must be remembered that analyses of such grain fractions obtained from among the flour fractions prepared by the miller do not necessarily show such high concentrations because of the partial mixing of the products from different parts of the grain which occurs within the mills. Later, Hinton (*Biochem. J.* **38,** *214* (*1944*)) showed that the scutellum of rye, maize, and barley has a similar high content of thiamine. It seems probable that this is true of all grains, including rice.

Nicholls has examined large numbers of grains from several samples of raw milled rice and parboiled rice and reports that the great majority of the grains of raw rice have a concavity marking the site from which the whole germ (scutellum and embryo) has been detached. On the other hand, the grains of parboiled rice do not show the concavity, but the site of the germ is flattened as though only part of the germ has been removed by milling. It seemed that parboiling had made the scutellum more ad-

herent, so that it had not been detached during milling.

Parboiling and also the new conversion process (parboiling first under vacuum and then under pressure) toughens the rice so that far fewer grains are broken during milling than with raw rice. This lowering of the brittleness would help the retention of the scutellum. It was concluded that parboiling probably does cause some movement of the vitamins within the grain, but that its chief value may be the retention of the scutellum.

## HIGH PROTEIN FEEDING IN EXPERIMENTAL BURNS

During the war extensive studies were made of the metabolic abnormalities following burns in man and in experimental animals and some of these investigations have been previously reviewed (*Nutrition Reviews* **3,** *2 (1945)*; **4,** *6 (1946)*; **5,** *46 (1947)*). It has been demonstrated that negative nitrogen balance, often with the loss of large amounts of nitrogen in the urine, regularly follows burns, usually lasts two to three weeks, and leads to a significant body protein deficit. A large loss of protein from the burn surface also occurs, further increasing the total protein deficit. By increasing the protein and caloric intake of food, presumably it would be possible to keep up with the surface loss, but during the so-called "catabolic period" which follows a burn, increasing the protein intake usually increases the urine nitrogen by an equivalent amount so that nitrogen equilibrium is difficult to attain (*Ibid.* **3,** *253 (1945)*). Forced feeding of a large amount of protein has in some instances achieved nitrogen equilibrium or slight positive balance. Such a program is often difficult to carry out and is usually poorly tolerated by the patient. Whether it is desirable to try to achieve nitrogen equilibrium soon after the burn by such forced feeding has therefore been questioned. To answer this question and to study the influence of testosterone and methionine in aiding the attainment of nitrogen equilibrium, F. L. Meyer, J. W. Hirshfeld, and W. E. Abbott (*J. Clin. Invest.* **26,** *796 (1947)*) report metabolic studies on 8 dogs before and after burning.

Of 4 animals force-fed, 2 became so ill that they had to be killed before adequate data could be obtained. The other 2 were given a fore period of force feeding which was also poorly tolerated although less so than after burning. Following the burn these 2 dogs were force-fed with the result that both were in positive nitrogen balance for the first five days, but in negative balance for the second five.

It was only possible to evaluate the results of testosterone administration in one of the 2 dogs so treated following burning. The authors believe that some retention of nitrogen was achieved over the controls, although no final conclusion could be drawn.

Methionine was given to 2 dogs for five days each following burning. No apparent effect on nitrogen balance was achieved, in contrast to the report of P. B. Croft and R. A. Peters (*Nutrition Reviews* **3,** *223 (1945)*) who found that methionine administration diminished the loss of nitrogen following burns in rats. However, as the authors point out, others have found methionine not to spare nitrogen following burns in man.

The increased food intake of the force-fed dogs was made up largely of protein without significant change in calories. The protein intake was increased by three times the control largely by the addition of a partial protein hydrolysate ("Amigen" and "Nutramigen"). Since large amounts of orally administered protein hydrolysates often lead to gastrointestinal disturbances it is not clear why the authors did not use natural, unaltered food protein. In fact, as the authors suggest, their conclusion that force feeding was poorly

tolerated must be qualified by understanding that the kind of protein used may have been responsible.

Combining their results with those previously reported in man by themselves and others, the authors suggest that it may be inadvisable to force feed patients in the first two days following the burn. Those who have treated burned individuals will certainly agree that it is almost impossible to force feed patients during the first few postburn days when fluid and electrolyte balance are becoming adjusted, and "toxemia" is at its height.

## ANTIMETABOLITES OF PYRIDOXINE

Derivatives and analogues of pyridoxine which are recorded as possessing antivitamin properties appear to be less numerous than antimetabolites related to other vitamins. However, two analogues of pyridoxine have been reported to be the most potent B-vitamin inhibitors yet discovered.

Both of these inhibitors possess alterations in the hydroxymethyl group in the 4-position ("para" to the nitrogen atom). One of these compounds was called desoxypyridoxine by W. H. Ott (*Proc. Soc. Exp. Biol. Med.* **61,** *125 (1946)*) who noted its high inhibitory activity. The other compound has been called methoxypyridoxine. Ott observed that desoxypyridoxine had an inhibition ratio of only 2 to 1 in the chick, i.e., 2 moles of analogue were required to counteract the effect of 1 mole of vitamin. Methoxypyridoxine was almost as effective, as reported by C. W. Mushett, R. B. Stebbins, and M. N. Barton (*Tr. N. Y. Acad. Sci.* **9,** *291 (1947)*). These observations were made on chicks, in which the action of the analogues seems to differ from the action on bacteria. In the latter case, E. F. Möller, O. Zima, F. Jung, and T. Moll (*Naturwiss.* **27,** *228 (1939)*) found desoxypyridoxine to possess somewhat less than 2 per cent of the activity of pyridoxine for Streptococcus plantarum.

Mushett, Stebbins, and Barton have also reported a study of pathologic changes resulting from growth reversal by these compounds, chiefly in the lymphoid and hematopoietic tissue of laboratory animals. In general, the changes were qualitatively similar to those observed with a pyridoxine deficient diet.

On a pyridoxine deficient diet, the ratio of spleen weight to body weight dropped from 0.23 per cent to 0.064 per cent in four weeks. When 50 micrograms of methoxypyridoxine or 100 micrograms of desoxypyridoxine per 100 g. of diet were fed, the spleen weight/body weight ratio was lowered significantly, and dropped nearly to the low level cited above when the desoxypyridoxine dosage was increased to 200 micrograms. In both the analogue-treated and the pyridoxine deficient chicks, the lymphoid elements of the spleen failed to develop. Similar results were obtained with dogs and monkeys, in which the spleen weight/body weight ratio was less than half as great in treated animals as in the controls.

Pups maintained on a pyridoxine deficient diet showed a progressive decrease in erythrocyte count, hemoglobin, and hematocrit values, and within a month developed a microcytic, hypochromic anemia. Pups receiving desoxypyridoxine likewise showed a microcytic anemia, but it was sometimes followed by partial recovery, and the blood values of one methoxypyridoxine treated animal were nearly normal. It was felt that the methoxy derivative possessed some pyridoxine activity for this species. Leukopenia developed within a month on both the deficient diet and the desoxypyridoxine treated group.

In addition to the changes in the spleen, monkeys treated with desoxypyridoxine (100 mg. per kilogram of body weight) suffered

atrophy of the thymus and lymph nodes, and developed microcytic anemia, leukopenia, and lymphopenia. Lymphoid elements diminished in the lymph nodes, particularly in the follicles, and in the adrenal cortex. The adrenal glands were enlarged, and inasmuch as the formation of lymphoid tissue is under control of hormones of the adrenal cortex, it was considered possible that the lymphoid atrophy might be due to stimulation of the adrenals by the vitamin analogue. Bone marrow also underwent atrophy; its color in desoxypyridoxine treated animals was pale yellow, and it was gelatinous in consistency.

Although comparisons among the various tissue changes were not made in monkeys on a deficient diet with those receiving the analogues, gross changes were observed which strongly resembled manifestations of pyridoxine deficiency. These included dryness of the hair, skin scaliness, tongue lesions, hyperirritability, and convulsions of epileptic nature. The two analogues differ in their effect on tryptophane metabolism since on a pyridoxine deficient diet rats treated with desoxypyridoxine and tryptophane excrete increased amounts of xanthurenic acid and kynurenine, whereas similar treatment with the methoxy analogue causes diminished excretion of these materials (C. C. Porter, I. Clark, and R. H. Silber, *J. Biol. Chem.* **167,** *573* (*1947*)). This latter effect (as well as the effect of the methoxy analogue on dogs) may possibly be due to a partial conversion of the analogue to pyridoxine. With these exceptions, however, the general paralleling of symptoms between analogue-treated and pyridoxine deficient animals makes it seem that desoxy- and methoxypyridoxine function specifically to compete with pyridoxine for the latter's combination in enzyme systems. The apparent lack of side reactions, such as those accompanying the use of sulfa drugs, lends promise to the possibility of using the pyridoxine analogues as therapeutic agents.

## NUTRITIONAL REQUIREMENTS OF PROTOZOA

The nutrition of protozoa is a subject which has received attention from relatively few investigators, although the practical importance of this field of study as it relates to malaria, leishmaniasis, kala-azar, syphilis, and other trypanosomal infections has already been emphasized (*Nutrition Reviews* **2,** *133* (*1944*); **5,** *284* (*1947*)). This lack of general attention is probably due in the main to the difficulty of culturing protozoa in mediums of known composition. Many of them have complex nitrogenous requirements (*loc. cit.*) so that blood serum or digest of, for example, liver or peptone, must be present for growth. Under such conditions a study of amino acid requirements is impossible. Nevertheless, the knowledge gained from protozoal studies should be of value to human nutrition, perhaps even more directly than that obtained with bacteria, due to the closer resemblance of protozoa to higher animals. Moreover, protozoa can be grown readily in an environment free from bacteria, so that conclusions reached as a result of experimentation need not be tempered by the possibility that bacterial synthesis may alter true growth requirements.

Tetrahymena cultures were the first among protozoa to be produced free of bacteria (A. Lwoff, *Compt. rend.* **176,** *928* (*1923*)). Others studied and reported over a period of years were several leishmanias, Schizotrypanum, and a number of trypanosomids (M. Lwoff, *Compt. rend. Soc. biol.* **128,** *241* (*1938*); **130,** *406* (*1939*)). Three trichomonads were studied by R. Cailleau (*Ibid.* **134,** *32* (*1940*)), and a fourth, Trichomonas vaginalis, has been discussed (*Nutrition Reviews* **5,** *284* (*1947*)). From

the information obtained, it appears that the trichomonads, leishmanias, and Schizotrypanum all require factors from blood serum, so that tests for vitamin and amino acid requirements have not been made. The trypanosomids were found to require thiamine, but synthesized cholesterol and ascorbic acid. Several strains were found to require hematin (G. W. Kidder, *Ann. N. Y. Acad. Sci.* **49,** *99* (*1947*)). E. G. Ball and co-workers (*Science* **101,** *542* (*1945*)), in a study of P. knowlesi, a malarial parasite, disclosed a number of unidentified growth factors together with a high para-aminobenzoic acid requirement for this organism.

Scattered information has been obtained concerning Paramecium and Colpoda. The former ciliate requires, according to W. H. Johnson and E. L. Tatum (*Arch. Biochem.* **8,** *163* (*1945*)) two unidentified factors from plasmolyzed bacteria, and later work by E. L. Tatum, L. Garnjobst, and C. V. Taylor (*J. Cell. Comp. Physiol.* **20,** *211* (*1942*); **21,** *199* (*1943*)) indicates that Colpoda requires thiamine, riboflavin, niacin, pantothenic acid, and pyridoxine.

Much information concerning protozoal nutrition has been obtained by G. W. Kidder and co-workers, in a series of studies with Tetrahymena, chiefly T. gelii W. This organism was cultured successfully in a medium free of unknown factors other than casein (A. P. Kline, *Physiol. Zool.* **16,** *405* (*1943*)) so that it became possible to study the need for individual amino acids (G. W. Kidder and V. C. Dewey, *Arch. Biochem.* **6,** *425* (*1945*); Kidder, *Ann. N. Y. Acad. Sci.* (*loc. cit.*)). The organisms, which under optimum growth conditions reached a population of over 300,000 cells per milliliter, were unable to grow at all in the absence of any of the nine amino acids essential for rat growth; omission of arginine permitted only feeble growth (6500 cells per milliliter). Omission of glycine resulted in virtually no growth when a mixture of the nineteen amino acids found in casein was employed, but when the medium contained only the ten essential amino acids, glycine had no effect. Kidder and Dewey interpreted this to indicate that glycine functions as a detoxifying agent for some of the non-essential amino acids, and suggested that glycine may fulfill a similar role in chick nutrition. Serine was found to be slightly stimulatory, and the final basal medium which was developed contained this amino acid in addition to the ten essentials. The total concentration of amino acids used was approximately 3.2 mg. per milliliter of medium.

T. gelii, was able to utilize d-tryptophane to a limited extent, since the racemic mixture was approximately three-fourths as active as the natural form. On the other hand, dl-leucine was only one-sixth as active as the l-isomer, indicating that the d-form was inhibitory. Cysteine exerted a sparing action on methionine, and homocystine replaced methionine completely in the presence of choline. Unlike lactic acid bacteria and Neurospora, this organism was unable to synthesize tryptophane from indole and serine. Finally, a mixture of serine, glycine, and cystine was able partially to replace valine.

In contrast to the amino acid requirements which paralleled those of mammals, T. gelii appeared to possess a greater ability to synthesize the various "B-vitamins." All of these except folic acid could be elaborated by the organism, although only para-aminobenzoic acid and inositol were produced in sufficient amounts to secure optimum growth. On the other hand, T. gelii resembled mammals in being able to utilize the polyglutamate forms of folic acid (G. W. Kidder and V. C. Dewey, *Proc. Nat. Acad. Sci.* **33,** *95* (*1947*)). Comparable tests of the activity of pteroyl mono-, tri-, and heptaglutamic acid indicated that half-maximum growth was obtained with 0.00064, 0.0005, and 0.00032 microgram per milliliter, respectively. The latter two concentrations were expressed in

terms of the pteroylglutamic acid content, so that the heptaglutamate was approximately twice as active as the monoglutamate. Apparently T. gelii stores and utilizes at least a part of the vitamin in the conjugated form, in a manner which may also be true of man (J. R. Totter, *Ann. N. Y. Acad. Sci.* **48,** *309* (*1946*)). In other microorganisms (for example, L. casei) the conjugated forms of folic acid are virtually without activity, indicating the lack of a specific vitamin conjugase which T. gelii evidently possesses. Fragments of the folic acid molecule, such as pteroic acid (PAB plus pteridine), pteroic acid plus glutamic acid, methyl pteridine or xanthopterin, were all inactive. A sample of the SLR factor possessed only 0.2 per cent of the activity of folic acid. Since the sample of SLR factor used was isolated from a natural source, it was thought that the activity might be due to contamination with folic acid.

T. gelii required purines and pyrimidines for growth. The purine requirements were met adequately by adenylic acid, and the pyrimidine requirements by cytidylic acid, uracil, or its nucleoside or nucleotide. Finally, an unidentified growth factor (V. C. Dewey, *Biol. Bull.* **87,** *107* (*1944*)) was discovered which was water soluble, light labile, adsorbed upon charcoal, and eluted by alkaline ethanol, and was not precipitated by salts of heavy metals. However, further reports dealing with the chemistry of this factor ("Factor II") have not appeared.

Experiments conducted to determine whether this organism could utilize ammonium salts to satisfy a part of the nitrogen requirement indicated that a reduction of essential amino acids to one-sixteenth the optimum level (36 micrograms per milliliter) was possible if an adequate supply of energy (2 mg. of glucose per milliliter) and 133 micrograms of ammonium pyruvate were also available. The ammonium iron thus appeared satisfactory for the synthesis of the nonessential amino acids. These experiments recall observations on the utilization of ammonium salts, not only by ruminants (S. Bartlett and K. L. Blaxter, *J. Agr. Sci.* **37,** *32* (*1947*)) but also in rats (D. Rittenberg, R. Schoenheimer, and A. S. Keston, *J. Biol. Chem.* **128,** *603* (*1939*)) wherein animals fed ammonium citrate containing $N^{15}$ were later found to possess the "tagged" nitrogen in some of their amino acids, particularly in glutamic and aspartic acids and arginine. The experiments were limited in scope, and were not designed to determine the kind or extent of amino acid synthesis which might be obtained with low levels of balanced protein. However, as Kidder points out, the observations deserve further study. It seems doubtful, on the basis of present knowledge that ammonium salts could be a significant source of amino acids in nonruminants, although a moment's reflection will serve to convince the reader of the importance of such an observation.

## RELATION OF ENDEMIC GOITER TO LACK OF IODINE

A provocative article by I. Greenwald (*J. Clin. Endocrinology* **6,** *708* (*1946*)) comes to the conclusion that endemic goiter is not due to a lack of iodine. The hypothesis that endemic goiter is due to iodine deficiency was presented by Prévost in 1849. This was based on the fact that physicians had used iodine successfully, and before the discovery of iodine had administered burnt sponge in the treatment of goiter. When Chatin shortly afterward presented the results of a large number of analyses of water, air, and food, a widespread use of iodine in the treatment of goiter was undertaken. The results were unsatisfactory due to overdosage with iodine until Baumann discovered iodine in the thyroid gland in 1896. This provided a new reason

for the use of the element. Subsequently the work of various investigators led to the prophylactic use of iodine in smaller amounts than had previously been used for therapeutic purposes.

Greenwald's review is a discussion of the data which have been published to date and adds no new data. It centers around the examination of three corollaries which he considers follow from the proposition (D. Marine, *Harvey Lectures* **19,** *96 (1923–1924)*) that "the immediate cause of the thyroid enlargement is a relative or an absolute deficiency of iodine."

The "corollaries" are: (1) Goitrous individuals should have low metabolic rates. (2) Goiters should contain less iodine than do normal glands, not only per gram of tissue, but per entire gland. (3) It should be possible (a) to produce goiter in animals by placing them on iodine-poor diets, and (b) to prevent it by the addition to such diets of such small amounts of iodine as are found in ordinary foods. That these three statements can be termed corollaries of Marine's hypothesis has been vigorously denied, however, by O. P. Kimball (J. *Clin. Endocrinology* **7,** *58 (1947)*).

A number of investigators have shown that the basal metabolic rate is usually within normal limits or possibly more frequently toward the lower range in simple goiter. This, of course, would be expected if the hypertrophied gland suffices to meet the demands for thyroid hormone.

Much of the information which has been published concerning the iodine content of enlarged glands is based on the concentration per unit of tissue rather than on the total content of these glands. Greenwald argues that the latter is the important correlation if one wishes to examine a hypothesis pertaining to lack of iodine, an argument again denied by Kimball (*loc. cit.*). The analysis by Greenwald of reports on nine hundred and sixty-nine thyroids, large and small, human, sheep, and dog, by seventeen different observers over a period of forty years, from Italy and Hungary to Japan and the United States does not show a negative correlation between the weights of the glands and their total content of iodine. Many of the data included in this tabulation were for normal thyroids, and therefore would seem to have little bearing on the argument at hand. Finally, it is not clear how the iodine content of a gland at operation or autopsy can be held to reflect the iodine content at the time of development of the goitrous condition.

As to the experimental production of goiters in animals, Greenwald notes that thyroid enlargements showing characteristic cytologic changes have been produced only on certain iodine-poor diets. Other diets appear to be ineffective. Moreover, to prevent such enlargements it has been found necessary to add much more iodine to these diets than is contained in some stock rations. To account for some of these findings he postulates that a positive goitrogenic agent exists in the diets on which goiter has been produced and that this agent is counteracted by the administration of iodine. It is surprising to find in such an extensive modern review no mention of the long-known and widely accepted role as goitrogenic agents of some foods and dietary factors such as cabbage, calcium, and thiocyanate (see E. V. McCollum, E. Orent-Keiles, and H. G. Day, "The Newer Knowledge of Nutrition," *Ed. 5, p. 255, The Macmillan Company, New York (1939);* W. T. Salter, *Ann. Rev. Biochem.* **14,** *570 (1945)*). The recognition of the existence of such relationships is but another example of the alteration of the requirements of one substance by the amounts of other factors in the diet—other examples of this relationship are iron and phosphate, thiamine and carbohydrate, tryptophane and niacin, protein and ascorbic acid. This fact alone in no way alters Marine's conclusion (*Harvey Lectures* **19,** *108 (1923–1924)*) that in simple goiter the cause of thyroid enlargement is a deficiency of iodine.

The data which Greenwald has reviewed concerning iodine intake and excretion in different regions also are interpreted as failing to support the thesis that the existence of endemic goiter in some areas is a direct result of an insufficiency of iodine. He considers the water analyses for iodine as unsatisfactory because the data on recovery of iodine have not been published in many instances or the recovery experiments were poorly designed. Compared with the amount of iodine obtained from the food or excreted in the urine, that furnished by even 1.5 liters of water per day is usually insignificant and most investigators have regarded the iodine content of the water only as an indication of the readily available iodine of the soil and, presumably, of the iodine content of the produce of that soil. Against this hypothesis, Greenwald cites analyses of two varieties of four vegetables which indicate that different varieties of the same vegetable grown upon the same soil and at the same time show greatly varying quantities of iodine. Although the differences which Greenwald cites are as great as those reported as existing between the mean iodine contents of vegetables or other foods in endemic and nonendemic regions, it is to be noted that he is considering single instances and not averages of several samples grown over a region. Greenwald recalls that R. McCarrison, C. Newcomb, B. Viswanath, and R. V. Norris (*Indian J. Med. Res.* **15,** *207 (1927)*) found no correlation between the iodine content of water and soil and the incidence of goiter in India.

Recognition of the difficulty of ascertaining the iodine intake from an analysis of food has led various workers to an examination of excretion of iodine in the urine. The validity of such an index of iodine intake depends on the assumption that in the adult who is in iodine equilibrium nearly all of the ingested iodine leaves the body in the urine or that the amount excreted through this channel is a good index of the iodine intake. There are, however, a few determinations of the iodine content of the feces in the literature and such as there are do not substantiate this assumption. It seems that the excretion of iodine in the feces is neither so small nor so constant as to be negligible in a consideration of the significance of differences in the excretion of iodine in the urine. It is also pointed out that, even aside from this point, none of the authors who consider their work as indicating that there is a difference in the iodine content in the urine in goitrous and nongoitrous areas seems to have published controlled analyses.

The iodine lack hypothesis has led to the use of iodine in prophylaxis. The prophylactic administration of iodine to man, however, has not reduced the incidence of new goiters to zero. In some regions, Greenwald argues there has been no effect at all or even an increase in the incidence. Kimball (*loc. cit.*), however, who directed the surveys from which Greenwald quotes, states that the inferences which he has drawn from them are "terribly wrong."

Greenwald concludes that there is no satisfactory evidence for the iodine lack hypothesis and that the history of goiter in many countries is that of a newly introduced epidemic disease which became endemic in some places with considerable temporal variation in severity. Hence, he holds that endemic goiter may not be a deficiency disease but is due instead to a biologic agent of unknown nature. In view of the most convincing demonstration that iodine administration can prevent endemic goiter (D. Marine and O. P. Kimball, *J. Am. Med. Assn.* **77,** *1068 (1921)*), the present review suggests that more logical conclusions are that the disease is to be regarded as due to an absolute or conditioned deficiency of iodine; that other dietary and physiologic factors may condition the organism's requirement of this element; that a minimal number of cases of "simple goiter" cannot be prevented by

iodine, a fact which indicates that thyroid enlargement may be produced by other methods than iodine lack; and, finally, that some of the evidence which has been cited for or against the iodine lack hypothesis is poor or unrelated to the question and should be disregarded or reinvestigated by proper laboratory and statistical methods.

Greenwald's review failed to consider the vast amount of recent work on antithyroid compounds. Inasmuch as many hundred such goitrogenic agents have already been described, it would appear most likely that some of these active substances may occur in foods. This already has been established in the case of the cabbage family, and the probability that positive goitrogenic agents might be the cause of the discrepancies noted in response to iodine must be considered. Thiocyanate goiter can be prevented by excess iodine, and consequently this would be a good example of a substance which would increase the iodine requirement. The other goitrogenic agents, however, are not inhibited by any amount of excess iodine. Greenwald's review would have been valuable had he proposed that lack of iodine is not the only cause of goiter. This would have been entirely correct, but his contention that iodine lack has nothing to do with goiter is entirely untenable.

## DENTAL CARIES EXPERIENCE AND GINGIVITIS IN POSTWAR ITALY

If the causes and control of oral lesions are to be fully understood, relationships between diet and tooth decay and between diet and gingivitis need further intensive investigation along both fundamental and clinical approaches. The present knowledge of nutrition and dental caries has been discussed (*Nutrition Reviews* **5,** *68* (*1947*)) as well as some aspects of nutrition and gingivitis (*Ibid.* **4,** *41* (*1946*)). The scarcity of factual material available in these two reviews indicates the inadequate knowledge of these fields. Therefore it is of special significance to consider the cooperative efforts of nutrition and dental investigators in clinical surveys of the type conducted in Italy in the early postwar period.

In 1945 the Italian Medical Nutrition Mission was sent from the United States to assess the effects of malnutrition on the Italian people. As a part of this survey, one team of workers studied the dental caries experience and the incidence of gingivitis in samples of the populace in four cities (Naples, Varese, Cagliari, and Catanzaro). In all, 3905 persons were examined. Those studied in Naples and Varese were from a low economic level and were unclean, underfed, and suffered from various undiagnosed chronic ailments. Those persons examined in Cagliari and Catanzaro were better fed and represented a higher economic level.

The rates of tooth decay in these groups have been reported by I. Schour and M. Massler (*J. Am. Dental Assn.* **35,** *1* (*1947*)). All examinations were made by means of mouth mirror and explorer in good natural light. The caries experience was obtained by counting the number of decayed, missing, and filled teeth (DMF). The average number of DMF teeth per person in the four Italian cities increased from 1.05 in the 11 to 15 year old group to 10.80 in the 51 to 60 year old group. These figures are low when compared with similar age groups in the United States where the average number of DMF teeth per person for the 11 to 15 year old and 51 to 60 year old groups was 4.66 and 23.20 respectively. Likewise there were considerably more individuals with no carious teeth in the Italian cities than in similar age groups in the United States. Attention should be drawn to the fact that these were the accumulated lifetime manifestations of dental

caries and not the increment in carious lesions during war years. The reasons for the lower prevalence of caries in Italy as compared with the United States were not clear. The people studied were relatively malnourished and oral hygiene was not commonly practiced.

Schour and Massler (*J. Am. Dental Assn.* **35,** *475 (1947)*) have described the gingival conditions found in the same individuals during the oral examination. It was felt that the gingivae of the lower anterior teeth provided a sufficiently adequate index of the general gingival condition of the oral cavity for this rapid type of survey. The severity of the gingivitis was graded numerically in four successive degrees (1+, 2+, 3+, 4+) according to increasing intensity and extent. These authors stated that their mildest classification of gingivitis (1+) "might be questioned by some clinicians who might prefer to include such very mild inflammatory changes within the range of normality. We prefer to consider this category as perhaps common and present in the 'average' person but nevertheless not normal and certainly not ideal." In addition, gingivitis was classified as acute or chronic as characterized by active or passive hyperemia, respectively.

The number of persons with gingivitis of all degrees increased from 40 per cent in the 6 to 10 year old group to 98 per cent in the 51 to 60 year old group. Not only did the incidence of gingivitis increase with age but the severity of both acute and chronic gingivitis increased with advancing age. In an examination of the percentage of persons examined with varying degrees of acute gingivitis, it appears that in the 6 to 10 year old group 32.4 per cent of these children had the mildest degree of acute gingivitis which, as mentioned above, might be classified by some clinicians as within the range of normalcy and only 7.7 per cent had moderate or severe gingivitis. Likewise in the 51 to 60 year old group 49.0 per cent of the group had the mildest evidence of acute gingivitis and 39.0 per cent had moderate or severe acute gingivitis. A somewhat similar distribution was observed in the classifications of chronic gingivitis among the various age groups. The consideration of these very mild and possibly questionable changes in the gingivae as gingivitis may account to some extent for the apparent extemely high prevalence of gingivitis among these groups. Comparable data are not known to exist about the incidence of various degrees of gingivitis in all these age groups in the United States. For American children up to 16 years of age, C. T. Messner, W. M. Gafafer, F. C. Cady, and H. T. Dean (*Pub. Health Bull.* **226,** *Washington D. C. (1936)*) and M. Brucker (*J. Dental Research* **22,** *309 (1943)*) have recorded a much lower incidence of gingivitis.

Unfortunately no reliable data appear to exist on the dental caries experience nor on the prevalence of gingivitis in similar age and population groups in Italy prior to the war. The opinion expressed to the survey team was that the number of patients going to the dental clinic of the University of Naples for treatment of gingival lesions had increased considerably during and after the war but that the incidence of tooth decay apparently had not been altered by wartime regimens. At best, these statements were personal opinions and cannot be considered as scientific evidence in either case. In connection with the gingival lesions, further surveys of comparable Italian population groups would be valuable to determine what effect improved nutrition in the postwar period had on these conditions. Of great significance would be specific experimental studies of nutritional therapy in test groups.

The low dental caries experience of the Italians in this survey is of interest to dental investigators. Since the lifetime caries accumulation was considered and not the increment in carious lesions during the wartime years, these data are not to

be compared with the dramatic reduction in the incidence of carious lesions observed in Norwegian children by G. Toverud (*Munnpleien* **28,** *4* (*Sept.*) (*1945*)). In the Norwegian children the reduction in incidence of carious lesions was directly or indirectly a result of the wartime diets. In the Italian population groups a low increment in carious lesions had existed throughout life. The problem confronted in this difference is of fundamental importance in assessing the extent to which heredity, environment, and dietary habits are responsible for tooth decay. G. W. Clapp (*Dental Digest* **34,** *320* (*1928*)) reported a low incidence of tooth decay in young adult Italians born in Italy and living in the United States who had maintained Italian dietary habits after moving to the United States. In contrast, C. D. M. Day and H. J. Sedwick (*Dental Cosmos* **77,** *442* (*1935*)) found an incidence of carious lesions in 500 children of Italian descent whose diet was presumably Americanized which was entirely comparable to the caries incidence of American children in general. These data indicate that the dietary habits are of greater significance than the hereditary influence although the latter may be of considerable significance when the dietary habits are borderline in caries-producing properties. Some information of the fluorine content of drinking waters from the various districts studied would be valuable. The object is the improvement of the diet without an increase in the susceptibility to tooth decay. This is entirely feasible as has been demonstrated in numerous experimental procedures (*Nutrition Reviews* **5,** *68* (*1947*)).

## SPECIES DIFFERENCES IN THE METABOLISM OF RIBOFLAVIN

P. B. Pearson and B. S. Schweigert (*J. Nutrition* **34,** *443* (*1947*)) observed that following ingestion of rather large amounts of synthetic riboflavin, cow's milk assumed a distinct greenish-yellow color characteristic of aqueous solutions of riboflavin. This suggested efficient transfer of ingested riboflavin to the milk. Microbiologic assay of such milk for riboflavin with Lactobacillus casei (E. E. Snell and F. M. Strong, *Ind. Eng. Chem.* (*Anal. Ed.*) **11,** *346* (*1939*)) showed varying degrees of increase in riboflavin content from none to a maximum of twofold. By standard fluorometric procedures (for example, M. L. Scott, F. W. Hill, L. C. Norris, and G. F. Heuser, *J. Biol. Chem.* **165,** *65* (*1946*)), however, the value found for riboflavin was approximately five times higher than that found microbiologically. This great discrepancy occasioned further investigation of the problem.

In goats and sheep Pearson and Schweigert (*loc. cit.*) reported that oral administration of 2 g. of riboflavin per day for three days increased the true riboflavin content of the milk collected during the last two days only about 26 per cent, as measured both by the microbiologic method and by a rat growth procedure. The milk of both species, however, contained a substance which behaved as riboflavin in the fluorometric test, which indicated increases of up to 500 per cent in the riboflavin content of the milk. This biologically inactive but fluorescent metabolite of riboflavin was also present in the urine of these animals, for which fluorometric assay indicated a content of riboflavin about twelve times higher than that obtained microbiologically. Before riboflavin ingestion, basal levels of riboflavin in milk and urine, as determined by either the fluorometric or microbiologic procedure, were in good agreement.

Extension of the tests to rats and human beings showed that values for the riboflavin content of urine, obtained microbiologically or fluorometrically, agreed both before and

after ingestion of riboflavin. In these species, therefore, the biologically inactive, fluorescent metabolite of riboflavin was not formed.

The chemical identity of this inactive metabolite of riboflavin was not established. All of the animals found to secrete it were ruminants, but it cannot be said at present whether the site of its formation was the rumen or the tissues. In connection with the former possibility, however, it will be remembered that J. W. Foster (*J. Bact.* **47,** *27* (*1944*)) isolated a microorganism from soil which, when cultured in the presence of large amounts of riboflavin, rapidly oxidized it to lumichrome. In any case, the investigation illustrates well the unexpected differences which occasionally are found to exist between species, and which should be borne in mind in extending results of studies with one experimental animal to another different species.

## ENVIRONMENTAL TEMPERATURE AND VITAMIN REQUIREMENTS

Considerable confusion and controversy have arisen over the claims of C. A. Mills and co-workers (*Nutrition Reviews* **1,** *283*, *426* (*1943*); **2,** *127* (*1944*); **3,** *187* (*1945*)) that in hot, moist environments, the requirements of rats for certain vitamins, specifically thiamine and choline, are increased, whereas the requirement for other vitamins remains constant.

Criticism of these conclusions has centered about the fact that Mills and co-workers add their vitamin supplements directly to the ration. Under such conditions, almost twice as much thiamine (in milligrams per kilogram of ration) is required to permit growth of rats at 91°F. as is required at 75°. Others (*loc. cit.*) have emphasized, however, that the food intake at the high temperatures is greatly reduced, and with it the thiamine intake; so that under the conditions of administration rats at the high temperatures are receiving considerably less thiamine than those at lower temperatures. When this factor is taken into consideration, and the absolute requirement of thiamine per rat per day is determined, slightly less thiamine is required by animals in a hot environment than by those in a cool environment (*Nutrition Reviews* **3,** *187* (*1945*)). This is the result which should be expected, since the thiamine requirement increases as the metabolic rate increases, and the metabolic rate increases at low temperatures.

C. A. Mills, E. Cottingham, and E. Taylor (*Am. J. Physiol.* **149,** *376* (*1947*)) review these observations and point out that, whereas the absolute thiamine requirement in micrograms per rat per day may be largely unaffected by temperature, in practice vitamins are normally ingested with the food, and their intake is thus affected by all factors which influence the amount of food eaten, for example, tropical heat, advancing age, and ill health. If, because of lowered food intake, a diet which is adequate at temperate climates becomes inadequate in tropical climates with respect to one essential nutrient, but not with respect to other essential nutrients, this is certainly important knowledge, and it will influence the dietary measures instituted to correct the situation. These workers therefore adhere to their previous approach, i.e., supplementation of the ration with various levels of the vitamins and ad libitum feeding to determine the influence of environmental temperature on the dietary requirements of chicks for thiamine, pyridoxine, niacin, folic acid, and choline. The same all-synthetic ration, with the appropriate vitamin varied and others present in excess, was used in all cases. Under these conditions, optimal growth of chicks in the cold (70°F.) occurs at a thiamine level of 1.0 mg. per kilogram of diet. At 90°F., 2 mg. of thiamine per

kilogram of diet are required for optimal growth. The polyneuritis threshold for chicks was 1 mg. per kilogram of diet at 70°F., and at 3 mg. per kilogram of diet at 90°F. No significant differences were found in chick dietary needs for folic acid, niacin, pyridoxine, or choline at the two different temperatures. Food intakes were measured, and were always somewhat less at the higher environmental temperature. Thus with chicks, the observations with respect to thiamine are the same as those with rats. In marked contrast are the observations with choline, the dietary need for which is unchanged at the two temperatures for chicks.

## RELATION OF CELL CALCIUM TO AGING AND CANCER

A. I. Lansing (*Science* **106,** *187* (*1947*)) has drawn together many reports to support a hypothesis that calcium, in the cell periphery or cortex, may be in some way correlated with the process of aging and cancer. A number of investigators were cited who found an increase of calcium in a wide variety of organisms with advancing age. Lansing (*Biol. Bull.* **82,** *385, 392* (*1942*)) had confirmed this and had found the marked increase to occur in the cell periphery or cortex. It was suggested that this accumulation may produce a decrease in the permeability of the cell membrane, which in turn may cause a progressive accumulation of toxic products of metabolism and the changes associated with aging.

The permeability and toxic product aspects of this picture are essentially theoretical and without sufficient experimental support, but the relation of calcium to longevity could be demonstrated experimentally. Lansing (*J. Exp. Zool.* **91,** *195* (*1942*)) was able to change experimentally the calcium content of rotifers by lowering the calcium content of the medium or by the addition of sodium citrate to remove calcium from the cell. This effect of citrate was confirmed by microincineration and electron microscopy experiments. Either technic of lowering cell cortex calcium resulted in marked increases in longevity of the rotifers.

A variety of reports has demonstrated that starvation increases longevity, and there is general agreement that the principal extension of life is due to prolongation of the growing period rather than the adult period. This appears true in Drosophila, in tadpoles, and in the rat experiments of C. M. McCay and associates (*Nutrition Reviews* **2,** *3* (*1944*)). It thus appears that it is the cessation of growth which initiates the aging process.

Since tumor tissue has an active and uncontrolled growth, one might expect to find a calcium situation opposite to that in aging tissue. Several references were cited to support the fact that calcium is reduced in tumors of epidermal origin in comparison to the normal skin tissue. The work of D. R. Coman (*Science* **105,** *347* (*1947*)) was mentioned in support of the possibility that the lowered calcium in tumors is in the cell surface. Coman found that normal cells can be more readily separated by micromanipulation in calcium-free mediums than in isotonic solutions, and that tumor cells are more readily separated than normal cells. Calcium deficiency in the cell membrane may thus favor easier separation of the cells.

Unpublished experiments of Lansing and associates reveal that the great calcium decrease in hyperplastic mouse epidermis and the resulting carcinomas is in the ultrafilterable calcium fraction. From these studies, Lansing has presented a single hypothesis to draw together the various facts reviewed. He postulates an organic calcium-binding fraction, probably protein in nature, existing in the cell cortex. This calcium-binding fraction is said to exert an

important function in the growth regulatory mechanism of cells. At the time of growth cessation this complex becomes altered in such a way that its calcium-binding capacity is increased, and thus during aging there is a continuing deposition of calcium. If, on the other hand, the calcium-binding complex is altered so as to decrease its base-binding capacity, then growth may continue. Extreme alteration of this complex may be the situation in the neoplastic state.

This is an interesting concept but requires much additional work to determine the nature of the calcium-binding compound, the changes which are postulated to occur, and what factors may produce these changes. The calcium differences reported in a variety of organisms do appear to be correlated with aging and cancer, and any well established correlations in the subject of growth and aging are important and demand detailed investigation.

## FOOD AND CAFFEINE STIMULATION

For centuries mankind has utilized caffeine and the related purine alkaloids as stimulants in the form of coffee, tea, maté, chocolate, and other beverages. The stimulating action of these naturally occurring compounds is more than psychic. The increased mental and physical efficiency or, at least, the relief from muscular and mental fatigue which follows the drinking of coffee or tea is the result of definite physiologic processes involving the central nervous system, the muscles, and the blood vessels. That the magnitude of the response to such beverages is modified by the simultaneous ingestion of food is a fairly common impression. Confirmation of the modifying influence of food is afforded by a study of the excitant effect of caffeine in rats (J. Haldi, W. Wynn, and C. Ensor, *J. Nutrition* **34,** *389 (1947)*).

Haldi *et al.* measured the spontaneous activity of control and test rats in cages suspended from a spiral spring in such a manner that even the slightest movements were recorded mechanically. In the first experiment young rats were given caffeine (75 mg. per kilogram of body weight) daily by stomach tube for three hundred and twenty experimental days. Litter mate controls were administered water during the same period. After one hundred days, spontaneous activity was measured daily during a two and a half hour control period followed by a four hour experimental period. Test solutions were administered at the beginning of the four hour period. Caffeine-fed animals showed a three- to fourfold increase in activity over the controls. This was most marked during the first thirty minutes but continued throughout the four hours. The effect of the caffeine disappeared within twenty-four hours and there was no augmentation of its action as a result of the prolonged daily administration.

In subsequent experiments adult rats were given 20 mg. of caffeine per kilogram, an amount which produced a definite stimulation of spontaneous activity if supplied in aqueous solution. The hyperactivity was markedly reduced when the caffeine was administered in sucrose solution (15 g. of sucrose per kilogram), in peptone solution (10 g. of peptone per kilogram), and in solution in vegetable oil (3.75 ml.). The modification of the action of caffeine did not appear to be due to a changed rate of absorption from the alimentary tract because the neutralizing action of sucrose was also evident in animals receiving the sugar by stomach tube and the caffeine by intraperitoneal injection. Furthermore, existing hyperactivity due to caffeine was greatly reduced by feeding the sugar solution thirty minutes after the oral administration of the chemical. The inhibition of hyperactivity

by sucrose, peptone, and fat was not the result of the mere presence of material in the alimentary tract because the stimulating effect of caffeine was unaffected by its oral administration in 10 per cent agar.

No explanation of the inhibitory effect of food on increased spontaneous activity in caffeine treated rats was proposed by Haldi *et al.* The restlessness of the animals may have been the result of a lowering of the threshold for transmission of impulses at nerve endings. P. Chauchard, H. Mazoue, and R. Lecoq (*Bull. Acad. de med., Paris* **129,** *456 (1945)*) have reported that the chronaxie of nerve fibers is affected by caffeine and that this effect is specifically neutralized by lactose, sucrose, galactose, and glucose. Haldi *et al.* believe that this opposing action is not limited to carbohydrates.

## EVALUATION OF PROTEIN HYDROLYSATES

Sufficient evidence has accumulated to indicate the therapeutic usefulness of protein hydrolysates in conditions of excessive nitrogen loss, such as after burns, trauma, and the shock of surgical procedures. A variety of protein hydrolysates are available, yet it is difficult to evaluate which of these may be the most efficacious in clinical use. Evaluation of such hydrolysates when employed at all, has been thus far restricted to the hydrolysate's ability to establish nitrogen equilibrium, or at best to its capacity to promote production of plasma protein. In the opinion of R. W. Wissler *et al.* (*J. Am. Dietet. Assn.* **23,** *841 (1947)*) these are only the minimum goals in the severely protein depleted patient; the objective should be to attain protein fabrication in other protein compartments of the body such as hemoglobin and vital organs, if not to replete protein reservoirs. These investigators feel that neither chemical nor microbiologic methods for the amino acids are sufficiently well developed and standardized for the evaluation of protein hydrolysates, and further that even if these are known, the requirements for maximal tissue synthesis after severe protein depletion are completely unknown.

The investigators suggest a "rat-repletion" method analogous to some vitamin assay methods. After establishing dietary protein depletion in rats, the results of a protein hydrolysate feeding may be evaluated in a period of two weeks. The depletion period may extend from one to three months, during which the adult rat has lost approximately 30 per cent of its weight, the serum protein has fallen from 4 to 5 g. per cent, and the hemoglobin from 10 to 13 g. per cent. After grouping the animals, the test rations incorporating the hydrolysate are fed for fourteen days. Three groups of control animals are recommended: (a) rats kept on the protein deficient diet, (b) a reference group fed an equal mixture of lactalbumin and casein, and (c) a group of normal rats maintained throughout the experiment on a 22 per cent casein ration. During the fourteen day repletion period the rats are weighed at designated intervals and the quantity of diet consumed is determined. At the end of the period the animals may be killed to determine hemoglobin levels, hemolysin titers, and liver and carcass protein. For simple screening tests, only the weight gains in relation to the amount of diet consumed were required.

The results are grouped according to the types of the twenty-five hydrolysates tested, and of the tabular data presented the "average nitrogen efficiency" (grams of weight gained per gram of nitrogen consumed) is of particular interest. Among the casein hydrolysates, acid hydrolysates failed to produce any weight gain, while enzyme hydrolyzed casein was very beneficial. Addition of tryptophane to the

acid hydrolyzed casein improved its value, while addition of methionine to an enzyme-hydrolyzed casein resulted in nearly the best of the hydrolysates tested. Enzyme-hydrolyzed lactalbumin was highly efficient. Of the other animal protein hydrolysates tested, enzyme-hydrolyzed beef plasma and liver gave high scores. Vegetable protein hydrolysates were in general not efficient, the best being a yeast product supplemented with both methionine and cystine.

Despite the nitrogen content and amount of diet offered to the animals being identical, very different amounts of diet were consumed by the animals. These differences are in accord with the previous experience of these investigators who noted that the food consumption drops markedly when a diet deficient in one of the essential amino acids is offered. In fact under the conditions of the experiment, the food consumption figures gave nearly as good a measure of the diet adequacy as the weight gains. The hydrolysate never reaches the efficiency of the unhydrolyzed protein, and the mixture of lactalbumin and casein (unhydrolyzed) promotes better growth than any of the hydrolysates. Enzyme-hydrolyzed protein is generally superior to acid-hydrolyzed. As mentioned by L. A. Maynard (*Science* **105,** *399* (*1947*)) the intact proteins appear to contain a growth principle different from the amino acids in their hydrolysates. This growth factor may reside in special groupings of amino acids possibly illustrated by strepogenin (*Nutrition Reviews* **4,** *273* (*1946*)).

Two hydrolysates termed A and B were studied in greater detail. The rats which received hydrolysate B barely maintained their weight, while those which received hydrolysate A gained well. The latter rats also showed a serum protein regeneration comparable to that of the control rats (fed lactalbumin-casein), although hemoglobin production was only half as great. In further comparisons of hydrolysates A and B, it was found that the group on hydrolysate A stored three times as much liver protein as the other group, and also formed three times as much antibody as measured by hemolysin output on the sixth day after injection of antigen.

The inefficiency of hydrolysate B was difficult to understand since it was stated to be an enzymatic digest of milk, beef, wheat, and yeast. According to the general observations of the investigators, such a hydrolysate should be highly efficient. F. J. Stare and G. W. Thorn (*J. Am. Med. Assn.* **127,** *1120* (*1945*)) have also pointed out that a commercial protein hydrolysate consisting of an enzymatic digest of milk, beef, wheat, and yeast does not support growth in young rats when it is fed as the sole source of protein. Rats receiving this preparation frequently have diarrhea and the same symptom is often observed in patients receiving this preparation in amounts such that nutritional benefits might be expected.

While it is true that these studies on evaluating protein hydrolysates for oral use in man have mostly been done on rats, and one must be careful in interpreting animal assay data in terms of man, nevertheless the observations strongly support the recommendation that protein hydrolysates must fulfill certain requirements of potency by a standardizing procedure, and that a hydrolysate incapable of producing fundamental physiologic responses in animals should not be offered as a desirable nutritional supplement for man.

## PRECURSORS OF NIACIN

The problem of disclosing compounds which may function as precursors of niacin has been the subject of considerable study and speculation (*Nutrition Reviews* **3,** *267* (*1945*); **4,** *334* (*1946*); **5,** *110, 234, 247* (*1947*)) mainly as a result of the findings that there

is an interplay between the utilization of tryptophane and niacin by various species, and that ingested tryptophane may lead to the excretion of N′-methylnicotinamide in the urine. The latter fact has suggested that tryptophane may be converted to niacin both in rats and man, although previous reviews have pointed out that the evidence supporting this concept is not direct.

Further information bearing on the tryptophane-niacin relationship in Neurospora has come from the work of G. W. Beadle and D. Bonner (*Arch. Biochem.* **11,** *319 (1946)*) and a later paper (G. W. Beadle, H. K. Mitchell, and J. F. Nyc, *Proc. Nat. Acad. Sci.* **33,** *155 (1947)*) points to kynurenine, a normal product of tryptophane metabolism, as an intermediate in the formation of niacin.

Using x-ray and ultraviolet irradiation, Bonner and Beadle obtained five strains of N. crassa which required niacin or nicotinamide for growth, in contrast to the wild type which grows well in the absence of the vitamin. Each appeared to differ from the parent strain by a single gene, as has usually been observed with x-ray Neurospora mutants (*Nutrition Reviews* **5,** *270 (1947)*), and it could therefore be assumed that failure of only one chemical reaction was involved in each. Tests indicated that the five strains represented three genetic types, and further studies were made with one from each group. These were designated as strains 3416, 4540, and 39401. It was expected that since each alteration resulted in the formation of a niacin requirement, at least three separate reactions were involved in the synthesis of the vitamin. The sequence was represented as $\rightarrow A \xrightarrow{1} B \xrightarrow{2} C \xrightarrow{3}$ niacin, with genes 1, 2, and 3 controlling the consecutive steps. The strain blocked at point 3 was predicted to accumulate substance C, active for strains 1 and 2, and the strain blocked at point 2 was expected to accumulate substance B, which should serve as a growth substance for strain 1.

These predictions appear to have been borne out by experiment. The three mutant strains were cultured for seventy-two hours in a basal medium with sufficient nicotinamide to permit fairly good growth, then filtered free of mycelium. When separate aliquots of this medium were sterilized and inoculated with each of the three strains, 4540 produced a substance which had niacin activity for 39401, but not for 3416 or itself. Concentration of the active material yielded two substances, both of which were isolated in crystalline form. Upon analysis, both were thought to be monocarboxylic acids, perhaps with an oxypyridine structure. One substance seemed to be a methylation product of the other. The oxypyridine structure was also discovered in N′-methyl-6-pyridone-3-carboxylamide, a metabolite of niacin in man (*Nutrition Reviews* **5,** *223 (1947)*) and a close relationship appears likely between the latter compound and those isolated from the mold.

Since the active materials from strain 4540 could not promote growth in 3416, the sequence of blocked reactions in Neurospora was considered to be $\longrightarrow A \xrightarrow{39401} B \xrightarrow{4540} C \xrightarrow{3416}$ niacin. Later, a reexamination of the growth requirements of strain 39401 (Beadle, Mitchell, and Nyc, *loc. cit.*) revealed that it could utilize either tryptophane or niacin, and it would seem therefore that the A → B step should be omitted from the scheme; the blocked reaction in this strain obviously lies ahead of tryptophane.

Further studies were made with 39401 and with 65001, the latter a strain which also utilized either tryptophane or niacin. Kynurenine was found active for 65001, providing half maximum growth at a level of 1.6 micromoles. Comparable growth was obtained with 1.4 micromoles of niacin or 9.8 micromoles of tryptophane, suggesting that kynurenine might undergo a nearly complete conversion to niacin. Niacin did not appear in the culture medium so long as the kynurenine was the limiting factor for

growth, but a fourfold excess of kynurenine resulted in the production of sufficient niacin to give maximum growth of strain 3416. The exact amount synthesized was not determined.

The experiments cited indicate strongly that Neurospora converts tryptophane to niacin, with kynurenine occupying a position ahead of the oxypyridine carboxylic acids described above. On the basis of present knowledge, the authors believe that the synthesis of tryptophane and niacin in Neurospora takes the following course:

→ anthranilic acid → indole $\xrightarrow{+\text{serine}}$ tryptophane

→ kynurenine → oxypyridine carboxylic acids → niacin.

Kynurenine, as Beadle and co-workers point out, thus assumes an importance in metabolism which has not heretofore been suspected. Moreover, the production of niacin in Neurospora appears to take a different course from the one in plants postulated by M. Guggenheim ("Die biogenen Amine," *p. 174, S. Karger, Basel (1940)*), wherein the precursors are thought to include ornithine, proline, delta-amino valeric acid, and arecoline and related compounds. These substances, as well as several known pyridine derivatives, possess no activity for Neurospora. Tests to determine the position of kynurenine, as well as the new acids, in the nutrition of mammals, will be awaited with interest.

## TOXICITY OF AMINO ACIDS AND AMINES FOR DEVELOPING SEEDLINGS

The surprising announcement that a number of compounds of amino acids and amines which are essential metabolites for plants exert toxic effects on the growth of seedling roots has been made by L. J. Audus and J. H. Quastel (*Nature* **160,** *222 (1947)*). While examining the effect of small concentrations of certain normal metabolites on seedling growth, it was discovered that a large number of these compounds have a distinct and in some cases considerable toxic action on the growth of cress roots.

The experiments were made according to a technic described earlier (*Nature* **159,** *320 (1947)*). Sterile cress seeds were grown under rigidly sterile conditions in a range of concentrations of compounds examined, together with controls in normal culture solutions. The various concentrations and controls were set up in triplicate samples of twenty seeds to the tube. After seven to ten days growth at 20 to 22°C. in artificial light for nine hours daily, the percentage germination and total weight of the roots produced were found for each concentration. From these values the "root growth index" defined as the fraction

$$\frac{\text{Average weight of roots per seedling in the presence of the inhibitor}}{\text{Average weight of roots per control seedling}} \times 100$$

was obtained. Graphs were then constructed relating these values to the concentrations of the substrates tested.

From the graphs it was easy to compile a table showing both the lowest concentration in parts per million at which inhibition is detectable, and the percentage inhibition of root growth at a concentration of 1000 p.p.m. Certain general conclusions could then be drawn. In the first place, only two normal metabolites examined among the amino acid and amine series showed no inhibition of cress root growth at low concentrations (1 part in 1000 or less). These were alpha-alanine and l-glutamic acid. These two acids are known to be highly active in transaminase systems (P. P. Cohen, *Biochem. J.* **33,** *1478 (1939); J. Biol. Chem.* **136,** *565, 585 (1940); Federation Proceedings* **1,** *273 (1942)*). They suggest the possibility

that other amine compounds may effect their inhibitions on root growth by competitive inhibitions in the normal transaminase systems of the plant.

Although glycine exerts a considerable inhibitory action, this was absent from hippuric acid (benzoyl glycine), suggesting that the amino group is essentially concerned in the inhibition. This suggestion is supported by results with the acetyl amino benzoic acids, in which the substituted compounds are less toxic than the parent amino acids.

The authors point out that tryptophane and tryptamine are known to produce physiologic reactions on plants similar to those produced by the auxins (F. Skoog, *J. Gen. Physiol.* **20,** *311* (*1937*)), and that although it has always been assumed that these effects are due to the occurrence of beta-indoleacetic acid in the samples, it is also likely that the compounds themselves exert a considerable toxicity.

Finally they remark on the interest of the finding that molecules such as niacin and urea have relatively strong inhibitory effects on seedling growth, and raise the question whether this can be due to the presence of basic groups in their molecules. This appears to be an example of the toxic effects of small concentrations of a normal amino acid metabolite, and it at once poses the question whether any similar activity may occur in animal nutrition.

## SIGNIFICANCE OF PLASMA PEPTIDES

The presence and significance of peptides in blood plasma have had a good deal of sporadic interest for about thirty years. Few fields have suffered as much from inadequate analytical procedures or yielded such discordant results. Thus far the results indicate that proteins may be absorbed partially as peptides and that large accumulations of peptides may occur in a variety of diseases. Peptidemia has been supposed to be characteristic of neoplasia and hepatic disease and as a postoperative or post-traumatic event.

The question has been carefully reinvestigated by H. N. Christensen and collaborators (H. N. Christensen and E. L. Lynch, *J. Biol. Chem.* **163,** *741* (*1946*); **166,** *87* (*1946*); H. N. Christensen, E. L. Lynch, and J. H. Powers, *Ibid.* **166,** *649* (*1946*); H. N. Christensen, P. F. Cooper, R. D. Johnson, and E. L. Lynch, *Ibid.* **168,** *191* (*1947*); H. N. Christensen, E. L. Lynch, D. G. Decker, and J. H. Powers, *J. Clin. Invest.* **26,** *849* (*1947*); H. N. Christensen *et al.*, *Ibid* **26,** *853* (*1947*)) who have provided methods for the determination of peptides in plasma. The method they describe begins with tungstic acid or trichloracetic acid precipitation of the plasma proteins. "Free" alpha-amino nitrogen is then determined upon the filtrate by the gasometric ninhydrin method of P. B. Hamilton and D. D. Van Slyke (*J. Biol. Chem.* **150,** *231* (*1943*)). The filtrate is then dialyzed, the dialysate hydrolyzed, and total dialyzable alpha-amino nitrogen determined. The difference between this and the free alpha-amino nitrogen is the conjugated diffusible ("peptide") nitrogen. This difference exists since deproteinized plasma may still contain considerable amounts of amino acid conjugates which are not dialyzable, hence not to be classified as diffusible peptides.

Using this method, very small quantities of "peptides" were found in normal plasma, usually less than 1 mg. of peptide nitrogen per 100 ml. Although increases were found in the plasma in some instances of disease, they were small, inconstant, and not related specifically to such conditions as neoplasia, hypertension, venous thrombosis, or hepatic disease.

The effect of the ingestion of milk and casein on the free and "peptide" alpha-amino nitrogen was studied. As expected, the "free" values rose, but in contrast the "peptides" decreased. No explanation for this strange phenomenon was offered.

Particularly interesting is the study of the amino acids and peptides in plasma and urine following the intravenous administration of partially hydrolyzed protein. Partially hydrolyzed casein and pancreas ("Amigen") were the first to be studied. "Amigen" is known to contain about one third of its amino acids in bound form. Within about two hours after infusion of "Amigen" into patients, the free alpha-amino nitrogen in the plasma had returned to normal, while the peptidemia persisted for about six hours, indicating a limited ability of the tissues to utilize these peptides. Loss of free amino acids in the urine following the infusion was from 2.4 to 6.0 per cent of that administered as free amino acids, while by contrast, 36 to 53 per cent of the peptides of the infused "Amigen" were excreted in the urine.

Two incomplete acid hydrolysates of fibrin were also studied, one containing about two thirds of the amino acids in conjugated forms, the other ("Aminosol") about one-third. Although the losses of free amino acids into the urine were similar for the hydrolysates of fibrin and of casein, the peptides of the fibrin hydrolysates were lost to a much smaller extent. The observations show that urinary loss of the peptides contained in protein hydrolysates may diminish appreciably the amino acids available to the patient.

## NUTRITION AND THYROTOXICOSIS

That the thyroid hormone is of great importance in regulation of the rate of metabolism in animals is well known. Hypofunction of the thyroid gland, however induced, results in a lowered metabolic rate and a host of sequelae which can be corrected by administration, in appropriate dosage, of desiccated thyroid or the amino acid, thyroxine. On the other hand, hyperfunction of the thyroid, or administration in excess of desiccated thyroid or thyroxine produces an increased metabolic rate, with accompanying symptoms and weight loss, which syndrome in man is referred to as hyperthyroidism or thyrotoxicosis. Because of the heightened metabolic rate associated with this condition, it would seem logical to expect an increased dietary requirement not only for calories, but also for certain of the biocatalysts (for example, vitamins) required in metabolic reactions.

These considerations have led several investigators to compare the requirements of thyroid-fed and normal animals for various vitamins, and to determine if some of the deleterious effects of thyroid feeding could be alleviated or eliminated by dietary means. Of the vitamins so far investigated, the evidence, although sometimes incomplete, indicates a heightened requirement during thyroid feeding for vitamin A (G. Logaras and J. C. Drummond, *Biochem. J.* **32,** *964* (*1938*)), thiamine (B. Sure and M. E. Smith, *J. Nutrition* **7,** *547* (*1934*); V. A. Drill, R. Overman, and J. H. Leathem, *Endocrinology* **32,** *327* (*1943*)), pyridoxine and calcium pantothenate (V. A. Drill and R. Overman, *Am. J. Physiol.* **135,** *474* (*1942*)), and niacin (E. Katzenelbogen, A. E. Axelrod, and C. A. Elvehjem, *J. Biol. Chem.* **141,** *611* (*1941*)). In no case, however, was it possible to eliminate the gross symptoms of thyroid feeding by administering pure vitamins. A few early experiments (for example, V. A. Drill, *J. Nutrition* **14,** *355* (*1937*)) and especially the later experiments of B. H. Ershoff and D. Hershberg (*Am. J. Physiol.* **145,** *16* (*1945*)) showed that inclusion of yeast concentrates or whole yeast in the ration resulted in better

growth and survival of rats fed desiccated thyroid than did heightened levels of the known vitamins.

Present availability of the newer crystalline vitamins and purified rations capable of supporting indefinite growth of rats has made further investigation of the relation of nutrition to thyrotoxicosis of interest. Ershoff (*Proc. Soc. Exp. Biol. Med.* **64,** *500 (1947)*) has compared the effectiveness of three diets in promoting growth and preventing the failure of ovarian development in young rats fed thyroid. Diet A was an all-synthetic ration containing sucrose 73.5, salts 4.5, and vitamin test casein 22.0. This was supplemented with high levels of thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin, choline, 2-methyl naphthoquinone, alpha-tocopherol, vitamin A-D concentrate, and sufficient cottonseed oil to supply essential fatty acids. In diets B and C, 10 parts of sucrose were replaced with 10 parts of dried brewers' yeast or of dried whole liver powder, respectively. All three diets supported equal growth of rats (average initial weight, 43 g.; final weight, about 200 g.), and equal ovarian development (average final weight, about 55 mg.), within experimental error, over the sixty day test period. The three diets were also fed with 0.5 part of desiccated thyroid replacing an additional 0.5 part of sucrose. At the end of sixty days the weight averages for the surviving animals of the three groups were 117 g. (diet A), 134 g. (diet B), and 202 g. (diet C). The corresponding ovarian weights were 24, 28, and 72 mg. One hundred per cent survival over the sixty day period was obtained when all three rations were fed without thyroid; with thyroid, 4 (diet A), 14 (diet B), and 15 (diet C), of the 20 animals initially in each group survived. The ovaries of animals receiving diet C with thyroid were normal functionally as well as histologically, since, when bred to normal males after the sixtieth day of feeding, such animals while still on the same diet gave birth to apparently normal young.

These results demonstrate the presence in liver of one or more factors which completely counteract the retardation of growth and the inhibition of ovarian development observed in young rats fed toxic levels of thyroid. The effect of liver could not be duplicated by doubling the vitamin levels in the ration, or by feeding singly the B-vitamins not included in it (inositol, para-aminobenzoic acid, folic acid, biotin). Only liver gave maximum protection at the level tested; although yeast gave some growth stimulation, it did not permit ovarian development at this level.

A similar study, with results which check closely with those of Ershoff, was made independently by J. J. Betheil, V. D. Wiebelhaus, and H. A. Lardy (*J. Nutrition* **34,** *431 (1947)*). In this study, the synthetic basal ration contained all of the known members of the vitamin B-complex, including folic acid, para-aminobenzoic acid, biotin, and folic acid, which were tested only singly by Ershoff. When 0.25 per cent of desiccated thyroid was added to this ration, significant retardations in growth were observed, as well as decreased survival, effects which could be largely counteracted by feeding dried whole liver or brewers' yeast. Liver was more than twice as effective as yeast on the weight basis. Yeast nucleic acid (0.5 per cent) exerted no beneficial effect. The effective factor(s) present in liver extract was stable to dry heat at 100° for twelve hours, and was not destroyed by autoclaving an aqueous suspension for one hour at 15 pounds pressure in the pH range from 1 to 10.

The results of these two investigations would seem to demonstrate the existence of a previously unrecognized water soluble substance, which, when present in ample amount in the diet, prevents certain of the harmful effects of thyroid feeding. Perhaps this new factor is normally required for growth of rats, but is supplied in necessary quantities either by intestinal synthesis, or

by synthesis in the tissues themselves. An increased requirement resulting from thyroid feeding may make these sources inadequate; conversely, the requirement may remain constant, but depression of the synthetic mechanisms by which it originally arose may occur, thus making dietary supplies of the substance essential. A third possibility would be that liver and yeast contain a substance which directly antagonizes the action of the thyroid hormone. This possibility seems less attractive in view of unpublished work, cited by Ershoff (*loc. cit.*), showing that liver does not counteract certain other manifestations of hyperthyroidism such as an increased basal metabolic rate or a hypertrophied ventricular wall. If effective in the human organism, such a factor would have obvious value in clinical practice.

## FECAL FAT OF NORMAL HUMAN BEINGS

The diagnosis of steatorrhea in human beings is a difficult problem, chiefly because of the lack of basic standards for comparison. Work has been undertaken to remedy this (*Nutrition Reviews* **5,** *51* (*1947*)) and another report by the same authors adds significant data to this neglected field (E. E. Wollaeger, M. W. Comfort, and A. E. Osterberg, *Gastroenterology* **9,** *272* (*1947*)).

In previous papers, these authors reported the results of studies of the total solids, fats, and nitrogen in the feces of 13 normal persons who were receiving a standard test diet that contained a large amount of fat (208 g. per day). This diet was suitable for detection of slight degrees of steatorrhea. Since patients with severe steatorrhea do not tolerate the ingestion of so much fat, a diet containing only a moderate amount of fat (102 g. per day) was prepared and intake-excretion studies were made on 11 normal subjects. The amounts of total solids, fats, and nitrogen in the feces were determined and the results were compared with those obtained by a study of normal persons who received the test diet high in fat.

Five men and 6 women, all normal, healthy, young adults, received the diet that was moderately high in fat. The procedures were the same as described in the earlier articles and the diet consisted of bland foods low in bulk, which were served in three meals and three between-meal feedings per day. Details of the menus were given in the paper. A large portion of the fat in both moderate and high fat diets was derived from milk. Other fats in the diet were derived from eggs and meats. In this study it was required that all food be eaten.

In the subjects who were receiving the diet moderately high in fat, the value for the total fecal solids varied from 13.6 to 39.1 g. and averaged 27.6 g. per day. The amount of fecal fat lost varied from 1.8 to 6.7 g. per day and averaged 4.1 g. In the subjects in which the high fat test diet was used, the amount of fat lost varied from 5.5 to 13.6 g. per day and averaged 8.7 g. Apparently, doubling the amount of fat ingested results in a corresponding increase in the amount of fat lost in the stools; therefore, the percentage of ingested fat lost in the stools was approximately the same with both diets; it averaged 4 per cent when the moderately high fat diet was used and 4.2 per cent when the high fat diet was used. On the other hand, the percentage of fecal solids which was fat differed rather markedly in the two series. In the series in which the diet was moderately high in fat, fat constituted from 9.3 to 19.6 per cent of the dried weight of the stool and averaged 14.5 per cent. In the series in which the diet was high in fat, the proportion of the total fecal solids which was fat ranged from 27 to 49 per cent and averaged 33.5 per cent.

The value for the fecal nitrogen in the normal persons who received a low fat diet

varied from 0.8 to 2.5 g. per day and averaged 1.7 g. There was no evidence from the data to indicate that fecal excretion of nitrogen was increased when the amount of fecal fat was increased. The calories lost in the stools also were calculated and it would appear that fewer calories were lost on the moderately high fat test diet than on the high fat test diet.

There is considerable evidence that an increased ingestion of fat results in the appearance of an increased amount of fat in the feces of animals (E. Hill and W. R. Bloor, *J. Biol. Chem.* **53,** *171* (*1922*); W. M. Sperry and W. R. Bloor, *Ibid.* **60,** *261* (*1924*)) and even more marked results have been obtained with human infants (L. E. Holt *et al.*, *J. Pediatrics* **6,** *427* (*1935*); A. Krakower, *Am. J. Physiol.* **107,** *49* (*1934*); and P. E. Rekers, J. C. Abels, and C. P. Rhoads, *J. Clin. Invest.* **22,** *243* (*1943*)). In an effort to determine the quantitative relationship between ingested and excreted fat, Wollaeger and associates constructed a figure by using the data obtained from two series of metabolic experiments reported by W. O. Atwater and F. G. Benedict (*U.S.D.A. Office Exp. Sta. Bull.* **136** (*1903*)), Benedict and R. D. Milner (*Ibid.* **175** (*1907*)), and by C. E. Wait (*Ibid.* **89** (*1901*)) in which the intake and output of fat, nitrogen, and carbohydrate were carefully measured. Ten normal human subjects were used in the experiment. The diets employed consisted of simple, easily digested foodstuffs. Each experiment extended over a period of one to six days and most experiments lasted three or four days. In the studies reported, the results of ninety-five experiments were tabulated.

From these studies, Wollaeger and coworkers have plotted fecal fat loss against dietary intake of fat and the upward slope of the resultant straight line shows a definite quantitative relationship between ingested fat and fecal fat. It is of interest to consider the bearing of such results in connection with the source of fecal fat in normal persons. It seems well established that at least part of the fecal fat is derived from sources other than unabsorbed dietary fat. Although Benedict ("A Study of Prolonged Fasting," *pp. 230–231, Carnegie Institution of Washington, Washington, D. C.* (*1915*)) expressed doubt that any feces are formed during complete fasting, other experiments (Sperry and Bloor, *loc. cit.*) showed rather definitely that even during fasting an appreciable quantity of fat is excreted in the feces. It is rather well accepted that the character of fat in the diet has little influence on the character of fat in the feces. From this it may be concluded that fecal fat is not derived to any great extent from unabsorbed dietary fat but rather it represents excretion into the bowel. Although several groups of workers have found that the iodine numbers and saponification values of fecal fat were widely different from the iodine numbers and saponification values of ingested fat, others have found the contrary. W. R. Bloor ("Biochemistry of the Fatty Acids and Their Compounds, the Lipids," *pp. 366–372, American Chemical Society Monograph Series, Reinhold Publishing Corporation, New York* (*1943*)) summarizes evidence relating to the source of fecal fat as follows: "... the lipids of feces represent excreted material to a great extent rather than unabsorbed food fat."

The observations of Wollaeger and coworkers and a critical analysis of studies reported in the literature indicate that the quantity of fat lost in the feces of normal human beings is influenced to a considerable extent by the amount of fat ingested. This work strongly suggests, although it does not prove, that unabsorbed dietary fat probably accounts for a larger proportion of fecal fat than is commonly supposed. There is considerable support for this belief in articles previously reviewed (*Nutrition Reviews* **5,** *155* (*1947*)).

It has been shown that fats as a class are well digested (more than 90 per cent of the

majority of fats are assimilated) although there are definite and consistent differences in their digestibility. K. F. Mattil has shown by statistical analysis that the chief limiting factor to digestibility of fat is the amount of saturated fatty acid of 18 or more carbon atoms which it contains (*Nutrition Reviews* **3,** *278* (*1945*)). Obviously, the more stearic acid the fat contains, the less digestible it will be and the more fat will appear in the feces. Since these studies deal with easily digestible fats commonly used for food, it would seem probable that the fecal fat of normal persons on an average diet is composed in good part of unassimilated fat of food.

These studies again point out that considerable accurate data frequently can be obtained by a critical review of good work which already has been published. It also points out that the human being is a complicated animal and any attempt to apply directly the results of animal experiments to human physiology is fraught with difficulties. Apparently, most studies suggest that a good portion of the fecal fat in man is the result of unabsorbed dietary fat. Since the amount of fat in the feces depends to some extent on the amount and the digestibility of the fat eaten, it would seem important to use standard test diets in which the amount and kind of fat and other foodstuffs are kept constant in order to detect small degrees of abnormal loss of fecal fat, such as may be encountered by the physician during clinical studies.

## FLUORIDES IN DENTAL CARIES INVESTIGATIONS

Various relationships of natural fluorides in public health have been described (*Nutrition Reviews* **4,** *227* (*1946*); **5,** *322* (*1947*)). A possible mechanism by which fluorides might effect a reduction of dental caries activity is in a reduction of acid production by oral bacteria or a decrease in growth of the organisms. B. G. Bibby and M. van Kesteren (*J. Dental Research* **19,** *391* (*1940*)) investigated the effect of various concentrations of sodium fluoride on the acid production and growth of various strains of oral bacteria. The acid production of fourteen strains of streptococci and twelve strains of lactobacilli was increasingly inhibited by sodium fluoride concentrations ranging from 2 to 1000 parts per million, but the inhibition at the lowest fluoride level was very small. This concentration corresponds to 0.9 p.p.m. of fluorine in drinking water. Concentrations in excess of 250 p.p.m. of fluorine were necessary to affect the growth of bacteria.

W. E. Clapper (*Proc. Soc. Exp. Biol. Med.* **65,** *333* (*1947*)) studied the effect of sodium fluoride on the acidogenic properties of eleven strains of lactobacilli isolated from saliva and maintained in peptonized milk agar and then tested for acid production by inoculation into flasks containing 100 ml. of tryptose phosphate broth containing 0.5 per cent glucose. Enough sodium fluoride was added to some flasks to give a concentration of 1 p.p.m. and to others enough to give a concentration of 100 p.p.m. Some flasks were kept as controls without any added sodium fluoride. The data indicated that sodium fluoride in concentrations of 1 p.p.m. and 100 p.p.m. inhibited acid formation from glucose by eleven strains of oral lactobacilli by averages of 8.8 and 11.4 per cent, respectively, at twenty-four hours, and by averages of 12.6 and 27.3 per cent respectively, at ninety-six hours. Resistance to the action of sodium fluoride on acid production varied among strains. However, growth of the organisms was not decreased by either concentration of fluoride so far as visual observation of turbidity indicated. This would seem to indicate that the processes of growth and cellular division had occurred despite the partial inhibition of acid production.

Samples of a single, strongly acid-producing strain of lactobacillus isolated from the saliva of an individual with rampant caries were grown for six months with weekly transfers in peptonized milk agar mediums containing 100 p.p.m., 1 p.p.m., or no sodium fluoride. At the end of this period, organisms from the three mediums were inoculated into flasks with no sodium fluoride present and their acid production determined by the method outlined above. Continued growth in the presence of 1 p.p.m. of sodium fluoride had had no effect on the acid-producing properties of this strain, but the sample which had been maintained in the presence of a concentration of 100 p.p.m. of sodium fluoride produced only 47 per cent as much acid as the comparable control. The original strain was again grown under the same conditions in the presence of 100 p.p.m. of sodium fluoride with a comparable reduction in acidogenic properties. In addition, the maintenance of this depressed strain on plain peptonized milk agar with no sodium fluoride for six months did not result in a return to the previous acidogenic properties.

The Thunberg technic was used to test the effect of sodium fluoride additions upon the activity of several dehydrogenases in one strain of oral lactobacilli. A suspension of lactobacilli was prepared from a culture of tryptose phosphate broth by centrifuging, washing with distilled water, and resuspending. A standard amount of inoculum was placed in the side arm of the Thunberg tube and tipped in after the tubes had reached a temperature of 37°C. The following substrates were tested: glucose, levulose, maltose, sucrose, lactose, glycerol, pyruvic acid, lactic acid, and formic acid. Concentrations of 1 p.p.m. and 100 p.p.m. of sodium fluoride were tested with each substrate. The time required to decolorize the methylene blue of the tubes with no added sodium fluoride was given a value of 100, while values for those with sodium fluoride additions were calculated as the percentage activity of the respective controls. All of the nine dehydrogenases except lactose dehydrogenase were inhibited by the presence of 100 p.p.m. of sodium fluoride. The presence of 1 p.p.m. did not inhibit any of the dehydrogenases but did stimulate the activity of lactose dehydrogenase and formic acid dehydrogenase. It seems unfortunate that the investigator did not select some intermediate concentrations which approximate the concentrations of fluoride in natural drinking waters in communities where the residents have a low dental caries experience or the concentrations which have been demonstrated to occur in enamel developed during exposure to fluorides.

S. B. Finn and D. B. Ast (*Science* **106,** *292 (1947)*) published preliminary data on the effect of the artificial addition of 1 p.p.m. of fluorine as sodium fluoride to the drinking water of Newburgh upon the oral lactobacillus acidophilus counts. In Newburgh, individual oral lactobacillus counts were made on random samples of the school population in 1944 and again in 1946 and 1947. In the city of Kingston which is being used as the control community in this experiment, random samples were taken in 1946 and 1947. Samples of saliva from 244 children of school age selected at random in Newburgh in 1944, prior to the addition of sodium fluoride to the public water supply, showed that 11.9 per cent were negative with respect to lactobacilli and 63.5 per cent had counts of 20,000 or over; early in 1946 after one year of fluorination, there were 15.4 per cent of the children with negative counts and 55.2 per cent with high counts; in 1947, 20 per cent had negative counts while 47.3 per cent had high counts. However, in Kingston where the fluorine content of the water is negligible, and where no sodium fluoride is being added 16.2 per cent of the children tested in 1946 had negative counts while 54.2 per cent had counts in excess of 20,000. In 1947 the distribution of counts was essentially the same as in 1946. Thus the percentage of

children with negative oral lactobacillus counts had increased in Newburgh between 1944 and 1946 and between 1946 and 1947 but had not increased between 1946 and 1947 in Kingston. Likewise the percentage of children with excessively high lactobacillus counts had decreased in Newburgh but not in Kingston. The standard significance tests had been applied to the differences between the 1944 and 1947 figures at Newburgh and had indicated that the differences observed were statistically significant.

It is of interest to compare the distribution of low and very high lactobacillus counts at Newburgh with the distribution in areas where fluorides occur naturally in the drinking water supply. H. T. Dean, P. Jay, F. A. Arnold, and E. Elvove (*Pub. Health Reports* **56,** *761 (1941)*) reported that in fluoride areas 37.4 per cent of the 12 to 14 year old children had negative lactobacillus counts and only 27.5 per cent of the same population group had very high counts. While the differences between 1944 and 1947 data at Newburgh are statistically significant, the reduction in percentage of high lactobacillus counts and increased percentage of negative counts do not approach the figures reported for natural fluoride areas. The short duration of the Newburgh experiment and the fact that the counts at Newburgh were on schoolchildren would indicate that any differences observed were largely related to postdevelopmental effects and not to developmental influences of the increased fluoride ingestion.

The effect of brushing the molar teeth of rats with powders containing fluorapatite has been studied by J. F. McClendon and W. C. Foster (*J. Dental Research* **26,** *233 (1947)*). The diet on which these rats were maintained was essentially that of C. A. Hoppert, P. A. Webber, and T. L. Canniff (*Ibid.* **12,** *161 (1932)*) with the exception that it had been modified to reduce the fluoride content. The molar teeth of the rats were brushed daily with powdered Tennessee brown rock phosphate. The 32 rats which had been maintained on this regimen from fifty-nine to one hundred days had an average of 0.16 carious teeth while the 32 litter mate controls whose teeth had not been brushed had an average of 3 carious lesions. Two similar groups of 58 rats each which were maintained for periods of from one hundred and three to one hundred and fifty-six days had averages of 1.25 and 4 carious teeth, respectively. The incisors of all animals whose teeth had been brushed with rock phosphate were bleached, indicating that sufficient fluorides had been unavoidably ingested to result in abnormal enamel formation.

An experiment in which 150 medical students participated for one year was performed to test the practicability of rock phosphate as human tooth powder. Forty students were given finely powdered Tennessee brown rock phosphate, which contains 71.4 per cent fluorapatite, to be used as a tooth powder daily. An additional 30 students were given an iron-free "synthetic apatite" consisting of hydroxyapatite with a surface layer of fluorapatite (total fluorine content of 0.25 per cent). At the end of the year the 40 students who had used rock phosphate had developed an average of 0.5 new cavities, the 30 using the synthetic product had developed 0.57 new cavities per man, and the 80 controls had an average of 1.5 new carious lesions.

To test the ability to eliminate fluorides McClendon and Foster personally ingested 2.5 g. of rock phosphate per day containing 77.5 mg. of fluorine for three weeks and then reduced the amount of rock phosphate to 1 g. per day (46.5 mg. of fluorine). One of the authors had two molars extracted prior to ingestion of rock phosphate and two molars extracted after ingestion of rock phosphate for six months. The dentin of the control molars contained 0.019 and 0.033 per cent fluorine, while the dentin of the molars exposed to high fluorine ingestion for six months contained 0.042 and

0.047 per cent fluorine indicating a definite and substantial storage of fluorine in the dentin. No bone storage figures are available but a higher storage would be expected in the bone than in the teeth. Excretion data indicated that of these high doses about 1.7 mg. of fluorine were retained daily. After a year of ingestion of 1 g. of rock phosphate daily by one of the authors, no bone abnormalities were observed by radiographic examination. However a year's ingestion of this amount of fluorine without evidence of toxicity would scarcely be considered adequate data, particularly if considered in terms of lifetime ingestion as might be the case using rock phosphate for brushing the teeth.

This series of papers indicates the interest which is prevalent in dental research at the present time regarding (1) the possible ways in which fluorides, in natural sources and added artificially, may be used in the reduction of the dental caries experience, and (2) the mechanisms through which the fluorides act in this reduction. Some investigators continue to minimize the toxicity of ingested fluorides. In various experiments man has been shown to have a high ability to excrete fluorides. When high amounts of fluorides were ingested in rock phosphate by McClendon and Foster, in the neighborhood of 1.7 mg. of fluorine were being stored each day, which constitutes an excessive amount of a toxic element which over long periods might result in toxic manifestations. The topical application of fluorides through the medium of tooth powders containing rock phosphate and the reduction in carious lesions is an interesting one but probably an impractical one for much of the material may be left accidentally in the oral cavity and subsequently swallowed.

## NUTRITIVE VALUE OF CASEIN AND LACTALBUMIN FOR MAN

Casein and lactalbumin are equally effective in the maintenance of nitrogen balance in the human adult according to A. J. Mueller and W. M. Cox (*J. Nutrition* **34,** *285* (*1947*)). This is not in accord with the results obtained in animal experiments in which lactalbumin has proved to be a superior protein, presumably due to its greater content of sulfur amino acids. This problem has been of particular interest to pediatricians because of the relatively larger proportion of lactalbumin in human milk compared with cow's milk. Few comparisons of the biologic value of casein and lactalbumin in man have been reported and it has been assumed that the results of animal studies are applicable to man. The superior biologic value of lactalbumin was evident in the early studies on rats by T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **20,** *351* (*1915*)) and this observation was extended subsequently to include dogs (H. B. Lewis, *Ibid.* **31,** *363* (*1917*)), mice (C. D. Bauer and C. P. Berg, *J. Nutrition* **25,** *497* (*1943*)), and chicks (H. J. Almquist, *Federation Proceedings* **1,** *269* (*1942*)).

The nutritive value of the two milk proteins was measured in balance studies on infants by F. Edelstein and L. Langstein (*Z. f. Kinderheilk.* **20,** *112* (*1919*)) who concluded that lactalbumin was superior to casein. Mueller and Cox have recalculated their data and find no significant difference in the biologic values of the proteins. H. E. Harrison (*J. Pediatrics* **8,** *415* (*1936*)) showed that adequate nitrogen retention occurred in infants fed a low intake of cow's milk proteins and H. H. Gordon, S. Z. Levine, M. A. Wheatley, and E. Marples (*Am. J. Dis. Child.* **54,** *1030* (*1937*)) found that similar nitrogen retention levels were attained in premature infants supplied equal amounts of nitrogen as cow's milk and as mother's milk. Contrary to observations on

rats and dogs, no improvement in the biologic value of casein hydrolysate was observed in children and adults as a result of supplementation with methionine (W. M. Cox *et al.*, *J. Nutrition* **33,** *437* (*1947*)).

Mueller and Cox have compared the two proteins in 4 healthy but slightly protein depleted adults, using nitrogen balances as the index of biologic value. The depletion diet supplied 40 calories and 8 mg. of nitrogen per kilogram of body weight. This nitrogen was supplied in the form of low protein fruits and vegetables. Little of this was apparently utilized since the fecal nitrogen during the control depletion periods always exceeded the nitrogen intake. Supplements of casein and lactalbumin were added at a level of 33 mg. of nitrogen per kilogram and amounted to 2 per cent of the total calories. The complete experimental period consisted of a twelve day depletion period followed by two four day periods of supplementation with each protein, with additional four day depletion or control periods intervening between the feeding periods. The subjects were in negative nitrogen balance throughout the experiment, the average daily losses of nitrogen during the four control periods amounting to 3.22, 3.39, 2.83, and 2.84 g. These negative balances were reduced to −0.79 and −0.68 by the feeding of casein and to −0.80 and −0.71 by the feeding of lactalbumin. Biologic values, calculated according to the method of H. H. Mitchell (*J. Biol. Chem.* **58,** *873* (*1924*)) were 89 and 95 for casein and 90 and 93 for lactalbumin. The species difference in the usefulness of the two proteins is illustrated by the fact that the gains in weight per gram of nitrogen ingested were 16.7 and 21.8 for casein and lactalbumin respectively, for rats fed similar samples.

These data strongly support the conclusion that the two milk proteins are of equal value for the maintenance of the normal adult and Mueller and Cox believe that similar results would be found in infants. This point requires experimental demonstration. If true, the clinical superiority of mother's milk over cow's milk may not be related to differences in the concentration of the two milk proteins.

Although the experiments just cited appear clearcut and decisive, caution should be used in their interpretation and application. Would it follow, for instance, that both proteins would be of equal value in metabolic stress due to infectious disease, severe hemorrhage, or caloric insufficiency? Is it possible that varying levels of dietary niacin and of choline may mask or exaggerate borderline intakes of tryptophane and of methionine, respectively? A complete understanding of the significance of a reduction of negative nitrogen balance as occurred in this investigation, is not yet possible. Possibly an inadequate protein, fed at the 33 mg. of nitrogen per kilogram level, would have yielded the same reduction in nitrogen balance. A. A. Albanese (*Advances in Protein Chemistry* **3,** *227* (*1947*)) has found that an intake of 100 mg. of nitrogen per kilogram is necessary to attain nitrogen equilibrium in normal adults fed low protein diets supplemented either with an enzymatic casein hydrolysate or an acid hydrolysate of casein enriched with tryptophane and cystine. Why is it that 33 mg. of supplementary casein nitrogen per kilogram will prevent 75 per cent of the nitrogen loss, as found by Mueller and Cox, if three times as much casein nitrogen is necessary to bring about equilibrium? Much more experimentation is required before these and other questions concerning the real significance of nitrogen balance may be answered.

# NOTES

## Chemical Relationship of Vitamins A and $A_2$

Several compounds closely related to vitamin A have been synthesized which have served to establish the formula of vitamin A (*Nutrition Reviews* **4**, *267* (*1946*)). The structural relations of vitamin A ($A_1$) and $A_2$ have been in dispute for several years, some believing that vitamin $A_2$ possesses a longer polyene side-chain than vitamin A, and others that vitamin $A_2$ has the same carbon skeleton but an extra double bond (see H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," *p. 70, Interscience Publishers, Inc., New York, N. Y.* (*1942*)). Recent evidence strongly supports the latter belief.

Related to the vitamins A are the retinenes observed by G. Wald, and these have been established as the aldehydes corresponding to the vitamin A alcohols (R. A. Morton, M. K. Salah, and A. L. Stubbs, *Biochem. J.* **40**, *lix* (*1946*)). Biologically the retinenes appear to be formed by the action of light on pigments in the dark adapted retina (G. Wald, *J. Gen. Physiol.* **22**, *775* (*1939*)). These relations may be summarized as follows:

$$\underset{\text{Vitamin A } (A_1)}{\text{Rhodopsin}} \xrightarrow{\text{light}} \text{Retinene}_1 \xleftarrow{\text{oxidation}}$$

$$\underset{\text{Vitamin } A_2}{\text{Porphyropsin}} \xrightarrow{\text{light}} \text{Retinene}_2 \xleftarrow{\text{oxidation}}$$

Morton, Salah, and Stubbs (*Nature* **159**, *744* (*1947*)) subjected vitamin A ($A_1$) to oxidation by aluminum tertiary butoxide (the Oppenauer reaction), using benzene as solvent and diethyl ketone as hydrogen acceptor. The product appeared to be retinene$_2$, that is, the aldehyde of the vitamin $A_2$ series. Retinene$_2$ was also prepared by manganese dioxide oxidation of vitamin $A_2$, and the crystalline 2,4-dinitrophenylhydrazone derivatives of this retinene$_2$ and that prepared from vitamin A by the Oppenauer reaction were identical. Retinene$_1$ was also converted into retinene$_2$ by the Oppenauer reagent, demonstrating that this reagent causes more than oxidation of the alcohol group. E. Haworth and co-workers (*J. Chem. Soc.* (*Part I*) *p. 128* (*1939*)) had previously isolated a $C_{20}$ aldehyde by oxidation of vitamin A by the Oppenauer reaction, although the product was not recognized as retinene$_2$, but evidence was presented that the aldehyde simply had an additional double bond in the beta-ionone ring.

When retinene$_2$ prepared from vitamin A was fed to vitamin A deficient rats, it gave rise in the lining of the small intestine and in the liver to a product showing the properties of vitamin $A_2$.

The experiments reported demonstrate that retinene$_2$ and retinene$_1$ must have identical carbon skeletons, and therefore those of vitamins A ($A_1$) and $A_2$ must also be identical. It appears then that the Oppenauer reagent may oxidize both the alcohol group and the beta-ionone ring, the latter by removal of two hydrogen atoms, and that an additional double bond of vitamin $A_2$ represents its only distinction from vitamin A. Details of the investigation and independent confirmation will be awaited.

## Survey of Food and Nutrition Research

A comprehensive survey of food and nutrition research currently active in the United States is being undertaken by the Food and Nutrition Board of the National Research Council with the support of the Quartermaster Food and Container Institute for the Armed Forces. This is a unique effort to compile a detailed record of research in progress in a special field which cuts across so many divergent interests.

The survey will cover all phases of food and nutrition research, including chemical, physiologic, microbiologic, technologic, psychologic, and basic economic studies. Cor-

relation of such studies with regard to health, acceptability, stability, utility, and economy of food will be made. Further information may be obtained from the office of the Food and Nutrition Board of the National Research Council, 2101 Constitution Avenue, Washington, D. C.

### A New Protein from Ovalbumin

K. Linderstrøm-Lang and M. Ottesen (*Nature* **159,** *807 (1947)*) report when working up "slops" of salt-free isoelectric ovalbumin solutions from half a year of experiments they observed that when the protein was crystallized with ammonium sulfate, it appeared as "beautiful rectangular plates instead of the usual flat cigar-shaped needles." At the time no observation of any infection of the solutions by bacteria or molds was made, but since urea was present and there was no antiseptic in the solutions, infection was a factor to be reckoned with even at the low temperature of 1°C. at which the solutions had been stored. Further sorting of their stock of old dialyzed ovalbumin solutions in the refrigerator revealed 6 samples of 1 to 2 years of age, 3 of which had been stored with toluene and 3 without. Only the latter were infected and upon crystallization they gave a 60 per cent yield of plates; all attempts to isolate plates from the uninfected solutions were unsuccessful.

This chance observation led the authors to consider it probable that some not very specific bacterial enzyme was active in the formation of the "plate" protein. The hypothesis was tested by using a dry powder of bacterial enzymes from Bacillus subtilis. Ten mg. of the dry powder, after acting for five hours on 1 g. of dialyzed ovalbumin at pH 6.5, converted the protein so far that 0.9 g. of the "plate" protein could be isolated from it, while at the same time only 1 per cent of the protein nitrogen had become soluble in 10 per cent trichloracetic acid. Thus it appeared that an almost complete transformation of one protein into another had been achieved. Such transformations are known in the field of enzyme chemistry, but it is of considerable interest that such a well known protein as ovalbumin can be enzymatically modified without serious degradation. The authors mention that they had already found crystalline trypsin devoid of activity, although as stated above, they could not expect it to be specific for the enzyme preparation they used.

The authors point out that the previous observations of C. F. C. MacPherson, D. H. Moore, and L. G. Longsworth (*J. Biol. Chem.* **156,** *381 (1944)*) upon ovalbumin "components" may or may not be closely related to the present work.

### Nutrients in Hormone Treated Tomatoes

During recent years tomato blossoms have frequently been sprayed with plant hormones to produce seedless fruit. A. D. Holmes, A. F. Spelman, J. W. Kuzmeski, and W. H. Lachman (*J. Am. Dietet. Assn.* **23,** *218 (1947)*) have studied the effect of this treatment upon the levels of various nutrients in tomatoes. Tomatoes were grown in a well fertilized plot under commercial greenhouse conditions during December to May. When the blossoms were fully open, they were sprayed with beta-naphthoxyacetic acid solution. This type of treatment has increased crop yields by about one-third, and produced a high percentage of seedless or virtually seedless fruit.

Comparative determinations of the content of water, calcium, magnesium, potassium, nitrogen, and sulfur in ten samples of hormone treated and control tomatoes revealed little differences. However, the treated tomatoes contained only 14.4 mg. ascorbic acid per 100 g., compared with 18.2 mg. in the controls. This decrease (21 per cent) in ascorbic acid content points to the need, frequently observed, for maintenance of nutrient quality along with improvements in gross appearance of fruit and vegetables.

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